J. Physiol. (1974), **240**, *pp.* 397–419
With 3 plates and 9 text-figures
Printed in Great Britain

THE MORPHOLOGICAL TYPES OF GANGLION CELLS OF THE DOMESTIC CAT'S RETINA

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(*Received 1 February 1974*)

SUMMARY

1. Three distinct morphological types of cat retinal ganglion cells have been identified and categorized as α, β and γ. Alpha ganglion cells have dendritic field diameters from 180 to 1000 μm; β, about 25 to 300 μm; γ, 180 to 800 μm, possibly more.

2. The dimensions of the α and β ganglion cell dendritic fields increase monotonically from the central area outwards to the periphery; those of the γ cells do not. Seemingly a spectrum of sizes of the γ cells is found at most locations in the retina.

3. All three morphological types of ganglion cells are found in the central area.

4. Possible further anatomical types of ganglion cells are discussed. Correlations are suggested between the morphological category α cells and the physiological class Y cells; between β cells and the X cells and between the γ cells and the W cells.

INTRODUCTION

It has been suggested, for those retinal ganglion cells with receptive fields organized into a centre with an opponent surround (Kuffler, 1952, 1953), that the dimensions of the dendritic fields are congruent with the centres (Gallego, 1965; Brown & Major, 1966; Dowling & Boycott, 1966; Rodieck, 1967); and that the surrounds are organized by the amacrine cells and the amacrine-amacrine synapses (Gallego, 1965; Dowling & Boycott, 1966; Rodieck, 1967). Now a great deal of new physiological data are available for the cat's retina which require a re-assessment of the evidence for the correlation between the dimensions of receptive field centres and the dendritic field diameters of the ganglion cells. Furthermore, attempts to give an anatomical basis to newly established physiological classes of

retinal ganglion cells have met with the difficulty that there is disagreement as to the morphological types of ganglion cell in the cat's retina (see, for example, the discussions in Enroth-Cugell & Robson, 1966; Creutzfeldt, Sakmann, Scheich & Korn, 1970; Ikeda & Wright, 1972*a*; Cleland, Levick & Sanderson, 1973).

Brown & Major (1966) thought the ganglion cells they described to be a single morphological type. Leicester & Stone (1967) recognized four or five different types and, in addition, a ganglion cell with a very small dendritic field diameter (15 μm) confined to the central area; Shkolnik-Yarros (1971) described at least seven morphological types of ganglion cells. All these authors used methylene blue and Golgi staining methods. Besides these descriptions there are ganglion cells which stain with reduced silver methods (Rushton, 1949) that have not been related to those stained by other methods. These sometimes seem to be regarded as a separate type (Gallego, 1965; Honrubia & Elliott, 1970). With the exception of these papers there appear to have been no published data, even in Cajal (1892, 1911), on the morphology of the cat's retinal ganglion cells.

Before 1966 only two physiological classes of ganglion cells were recognized in the cat's retina. These were on-centre and off-centre cells with surround opponency as initially defined by Kuffler (1952, 1953). In 1966 Enroth-Cugell & Robson described a further dichotomy in the physiological classification of ganglion cells which could be superimposed upon Kuffler's on-centre, off-centre classification. They referred to the two new classes of ganglion cells as X and Y cells. Cleland, Dubin & Levick (1971) confirmed and extended the classification, emphasizing the sustained (X) and transient (Y) nature of the ganglion cells' responses. These authors also established a correspondence between the Enroth-Cugell & Robson classification and the slower and faster conduction velocity groupings of the fibres in the optic pathway to the dorsal lateral geniculate nucleus. Independently this identification was supported by Fukada (1971) and Fukada & Saito (1971), who referred to the groupings of the dichotomy as Type 1 (Y) and Type 2 (X) cells.

Stone & Fabian (1966), Rodieck (1967), Fukada (1971) and Cleland *et al.* (1971, 1973) all reported cells which did not fit the Enroth-Cugell & Robson (1966) classification. Within the last two years Stone & Hoffmann (1972) and Hoffmann (1973) have given further evidence for cells with rather varied receptive field characteristics, such as 'suppressed' or 'excited' by contrast and directional selectivity. These cells have slower conduction velocities than the X and Y cells and, mainly based on this criterion, Stone and Hoffmann introduced them as a new physiological class of retinal ganglion cell called W cells. Thus there are at least three new physiological classes of cells, besides the two classes originally established

by Kuffler, to correlate with the morphological types to be described in this paper.

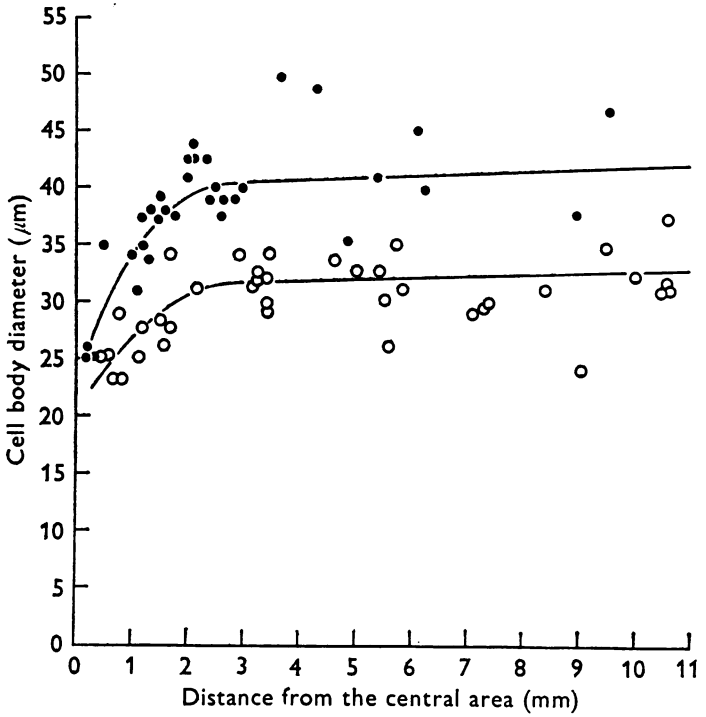
The first part of the results section gives qualitative definitions of the morphological types of retinal ganglion cells in the cat's retina and compares them with those of previous authors. The second part provides quantitative information based on the decisions taken in the first section.

METHODS

The retinae studied were from the eyes of adult cats and were those obtained and prepared as described in Boycott & Kolb (1973*a*) and B. B. Boycott (in preparation). Of the 250 or so retinae used in those papers, only three, all Golgi–Cox stained retinae, had a sufficiency of stained ganglion cells whose position could be defined relative to the central area. All the numerical data, except for those of the cells in the central area, are taken from these three retinae. The Golgi–Cox preparations never revealed stained cells in the central area; for these cells data were obtained from two Golgi-rapid processed retinae. Recognition of the cell types to be described here is primarily dependent on examination of flattened whole mounts of the retina. The morphology of the dendrites of the ganglion cells cannot be observed with adequate accuracy by the exclusive use of vertical sections, and reliable determination of their position relative to the central area is also more difficult. Our morphological identifications are based on Golgi–Cox material, but we have been careful to identify all the types in Golgi-rapid and Golgi–Colonnier processed retinae. We found some differences of detail between the methods but no evidence for further morphological types. Because whole mounts were used, the level of branching of the ganglion cell dendrites in the inner plexiform layer could not be systematically determined.

Criteria for the dimensions and position of the cat's central area are given in Bishop, Kozak & Vakkur (1962), where the average distance from the centre of the optic disk to the central area is given as 3·42 mm. In our whole-mount preparations stained ganglion cell axons could be seen at low power to arch from an apex at the blind spot outwards on either side of the central area. The symmetry of that pattern permitted easy location of the central area at a distance of about 3–4 mm from the blind spot. A more exact identification of the central area was obtained by putting the condenser out of focus, or inserting the substage phase ring, so that the outlines of the perikarya of the unstained cells could be seen. Quantitative positioning of the central area from the cell densities described in Stone (1965) was not possible, but moving the slide around by more than about 0·5 mm in any direction readily showed when there was a decrease of cell density. Assuming from Stone's (1965) data a total diameter of about 1 mm for the central area, we think that we identified the centre of the central area to within about 300 μm. Thus zero represents the centre of the central area and cells indicated on the graphs within 0·5 mm of that point are cells within, or on the border of, that area. There are no available comparative data on the shrinkage or swelling of retinal tissue processed by Golgi procedures and we have attempted no allowance for this, or the distortions produced by flattening during mounting on the slide. When the central area had been found the Vernier co-ordinates of the Zeiss mechanical stage were taken, then the co-ordinates for an individual cell. By using Pythagoras's theorem the distance of the centre of the perikaryon of a cell relative to the central area was found. The error of measurement was about $\pm$ 50 μm. The dimensions of the cells were taken with a focusing micrometer eyepiece. The perikarya were measured at their maximum optical section, in ortho-

gonal directions, thus giving a maximum and minimum diameter of which the arithmetic mean was used. The same procedure was used in the measurement of the dendritic fields. Irrespective of the type of the cell, many of the dendritic fields of the ganglion cells varied from circular to ellipsoidal. The major and minor axes usually differed by between 10 and 20%; occasionally the difference was as much as 30%. The perikaryon was not always positioned in the exact centre of the dendritic field of a cell.



Text-fig. 1. Perikaryal diameter of α-type ganglion cells as a function of distance from the central area. ●, cells stained with the Golgi-rapid method. ○, cells in another adult cat stained with the Golgi–Cox method. The lines were fitted by inspection. For discussion of the data see below.

In the Results section three types of ganglion cells are described and designated α, β and γ. Their discrimination has its basis in the recognition of differences in the form (morphology) of the branching pattern of the dendrites. This qualitative assessment of the cell types was followed by measurement of the dendritic field and perikaryal diameters of the three types. All cells were specified with respect to their position relative to the central area. Comparisons can be made between the dendritic branching pattern as observed with the methods we have used and the morphologies described by previous authors because these features are less dependent on the methods of fixation and preparation of the retinae. The diameters of the dendrites and the dimensions of the perikarya are less easily compared with those of other authors because such dimensions are more dependent on the methods of fixation, post-fixation processing procedures and the method of staining. Different Golgi procedures were found to have different effects on the swelling or shrinkage of cells.

Because the Golgi-rapid and the Golgi–Cox methods gave the same results for the branching patterns of the dendrites and the diameters of the dendritic fields, we could recognize and compare α cells from the two procedures. As can be seen in Text-fig. 1, the perikaryal dimensions at equivalent retinal eccentricities differed consistently between the two methods. The Golgi-rapid preparation gave perikaryal dimensions a mean factor of about 1·3 greater than those obtained using Golgi–Cox. Adequate numerical information for a comparison of the β and γ types of ganglion cells with the Golgi–Cox stained equivalents could not be obtained in Golgi-rapid material due to an insufficiency of stained cells and retinae without damaged central areas.

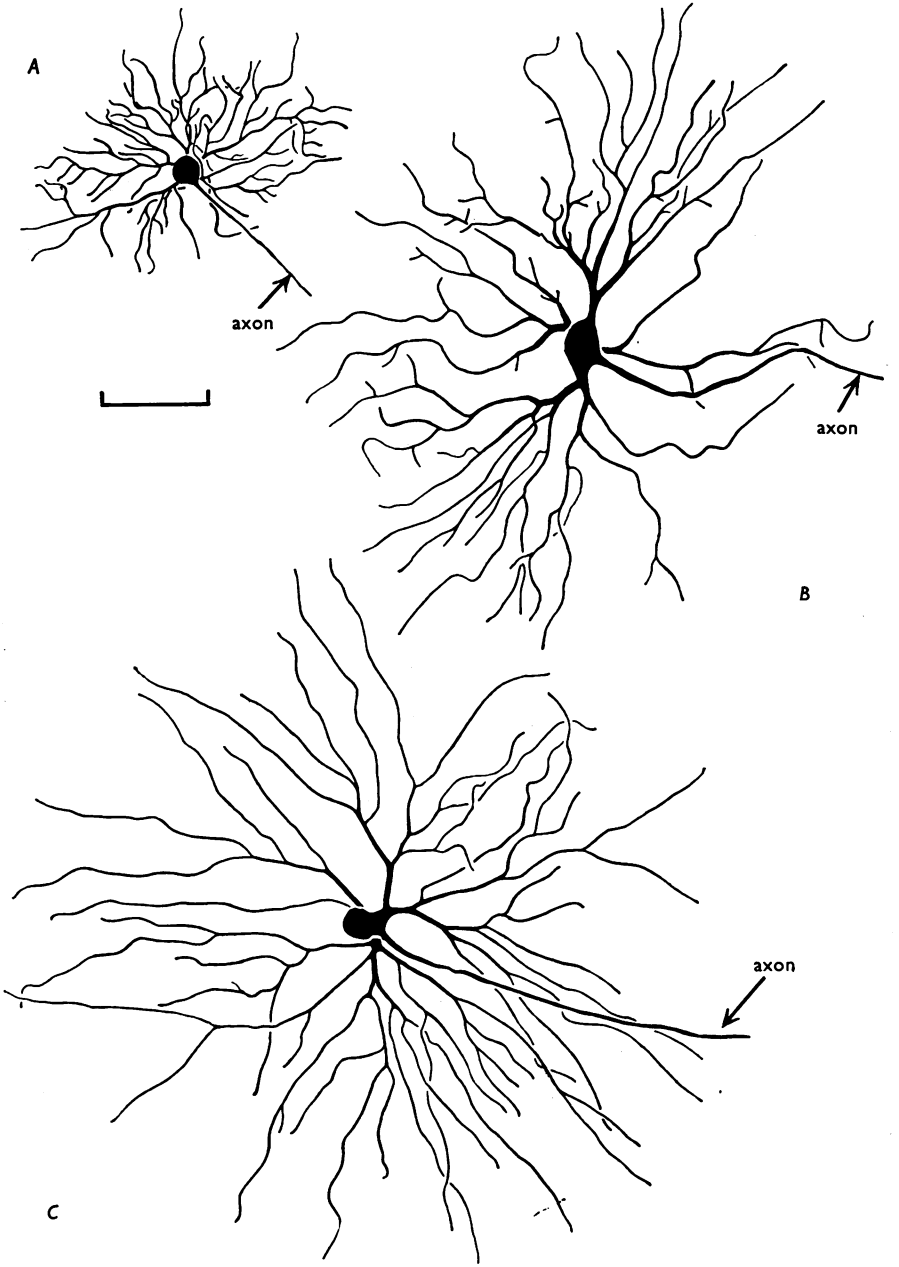
As far as we can discover there is no previous report of a difference of this kind in the reaction of comparable cells to two different Golgi procedures. The effects were similar for the other classes of ganglion cells. Differences produced by preparative procedures must be taken into account should the sizes given here be compared with those obtained by other methods; especially with dye-injected material where the perikaryal size might form a major classificatory feature.

RESULTS

General description of the ganglion cell types and their relationship to previously published categories

(*a*) *α and β ganglion cells.* Brown & Major (1966) described ganglion cells they considered to constitute a single morphological type with a bimodal distribution of dendritic field diameter. For the reasons given below we consider these to be two distinct morphological types. Their larger cells are referred to here as α cells and the smaller ones as β cells.

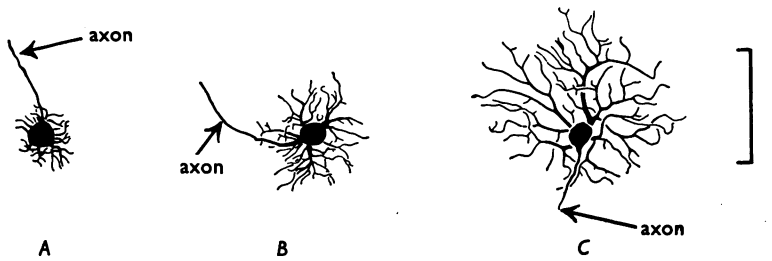
The α cells illustrated in Text-fig. 2*A–C* are respectively located 1·2, 8·3 and *ca.* 10·0 mm from the central area. Pl. 1 is a photomicrograph of an α cell 7·4 mm from that area. Those α cells near the central area (Text-fig. 2*A*) have primary dendrites which are thinner than those of cells of the same class towards the periphery. The α cells, at least away from the central area, generally have from three to six primary dendrites. When the primary dendrites branch the dendritic diameter approximately halves at the first few branch points, after which, at succeeding points, the dendritic branches remain at an approximately constant diameter as they go relatively straight towards the periphery of the cell's dendritic field. Generally on these finer dendrites such branches as are present are relatively short. Approximately speaking the thicker dendrites of the α cells pass vertically or obliquely through the inner plexiform layer, and the thinner dendrites are on a horizontal plane within that layer; frequently they are at levels near the inner nuclear layer. Text-fig. 2*A–C* and Pl. 1 show the over-all appearance of the α-type ganglion cell, as a cell with rather sparsely branched dendrites going relatively straight out radially from a large perikaryon. This feature is also well shown in the diagrams of methylene blue preparations in both Brown & Major (1966) and Leicester & Stone



Text-fig. 2*A*, *B*, *C*. Three α-type ganglion cells, their distances from the central area were: *A*, 1·2 mm; *B*, 8·3 mm; *C*, about 10·0 mm. These and the following drawings were made by transferring measurements taken with a focusing eyepiece micrometer on to graph paper. All drawings are from Golgi–Cox material. All the scales represent 100 μm and all the diagrams are reproduced at the same magnification.

(1967). We estimate from our data in Text-fig. 7 that their cells were about 3 mm from the central area. Although the similarity of the cells as revealed by the two methods of staining is striking, there may be some slight differences of detail in that our cells seem to have a few more small branches. However, as Cajal (1892) pointed out, methylene blue often does not stain the finer branches of nerve cells.

Text-figs. 3*A–C* and Pl. 2, fig. 2, show β-type cells 1·2, 2·9 and 10 mm distant from the central area. They are reproduced on the same scale as those in Text-fig. 2. At equivalent positions in the retina they are smaller in all respects than the α cells; this is clearly shown by the example in Pl. 1. That plate also shows that when the cells are seen near each other, the subjectively estimated differences in the dendritic branching patterns are very evident. At present we have not thought it necessary to undertake the very considerable task of attempting to quantify the differences in



Text-fig. 3*A*, *B*, *C*. Three β-type ganglion cells, their distances from the central area were: *A*, 1·2 mm; *B*, 2·9 mm; *C*, 10·0 mm. Text-fig. 3*B* is a drawing of the β cell in Pl. 2, fig. 2. Comparison of the Text-figs. 3*A* and 2*A*, which are both at the same distance close to the centre of the central area, shows that the two cell types are clearly discriminable. A comparison of the peripheral cells 3*C* and 2*C* at 10 mm from the central area shows that they too are clearly recognizable as different. But comparison of 3*C* and 2*A* shows that, without information as to the degree of retinal eccentricity, inappropriate classifications could be made.

branching pattern of the α and β cells, which would, of course, have to be done at chosen points across the retina. However, by inspection, at equivalent retinal locations, β cells have more branches per unit area of dendritic field than the α cells.

Comparison of our β cells with the smaller of the two populations of ganglion cells described by Brown & Major leaves no substantial doubt as to their correspondence. There is, as with the α cells, the detailed difference that there are seemingly more fine dendritic branches on our cells.

Comparison of the central α cell of Text-fig. 2*A* with the peripherally situated β cell of Text-fig. 3*C* shows why we have been careful to identify

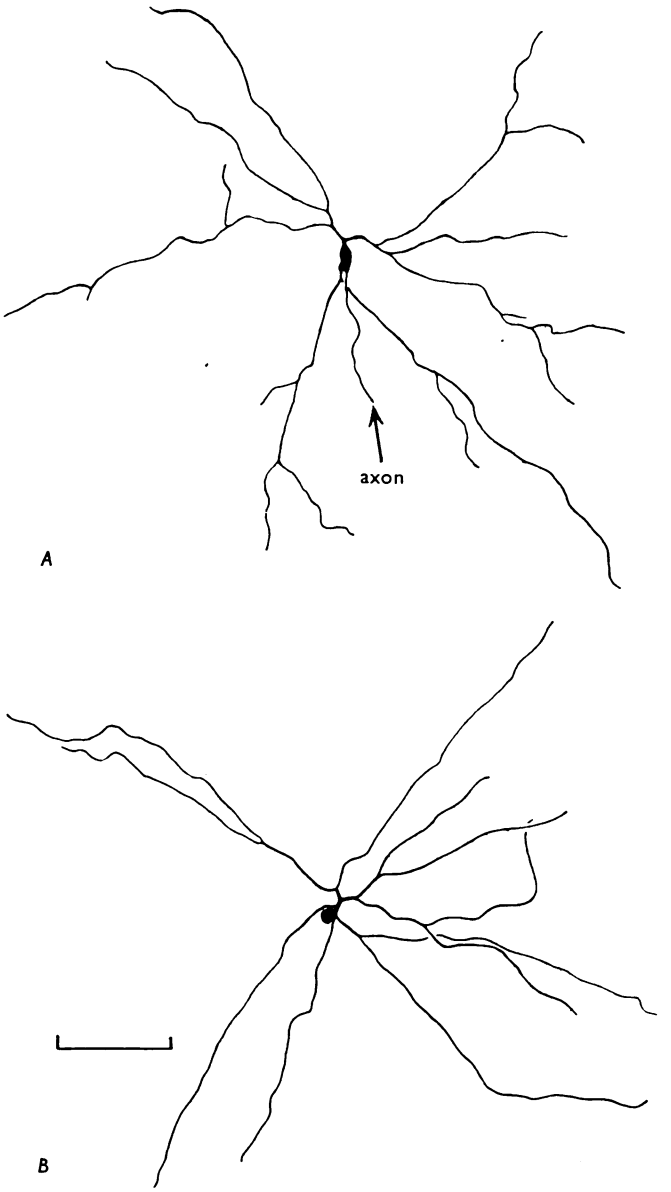
all our different morphological types of ganglion cells at equivalent positions in the retina. If they are compared without information as to their degree of retinal eccentricity or size, the morphology of these two cells is very similar. Given such information α and β cells are always distinguishable (Pl. 1). It is strongly to be emphasized, when the ganglion cell types described here are identified by other methods – for example, by the injection of dyes – that their position relative to the central area is an essential additional parameter of identification. A similar problem has recently been analysed for the horizontal cells of the primate retina (Boycott & Kolb, 1973*b*).

Leicester & Stone (1967) described two classes of cells having a single dendrite with 'loosely' or 'densely' branched dendritic fields and also a third class of shallow multidendritic cell. The dendritic spreads and perikaryal diameters are compatible with cells in our β category. Within the β category we find cells with single primary dendrites, two or more and, in the periphery of the retina, with up to six primary dendrites.

(*b*) γ *ganglion cells*. Leicester & Stone (1967) briefly mentioned, without illustration, four cells each with a small perikaryon and a large dendritic field. Shkolnik-Yarros (1971, her Figs. 13 and 14*B*) also observed cells with small perikarya and large dendritic fields. We have observed many cells of this kind with the general form shown in Text-figs. 4*A* and *B*; 5*A* to *C*; Pl. 2, fig. 1 and Pl. 3, fig. 1. Although there is evidence (page 412) that they may be a less homogeneous population than the α and β groupings, they will be collectively referred to here as γ cells.

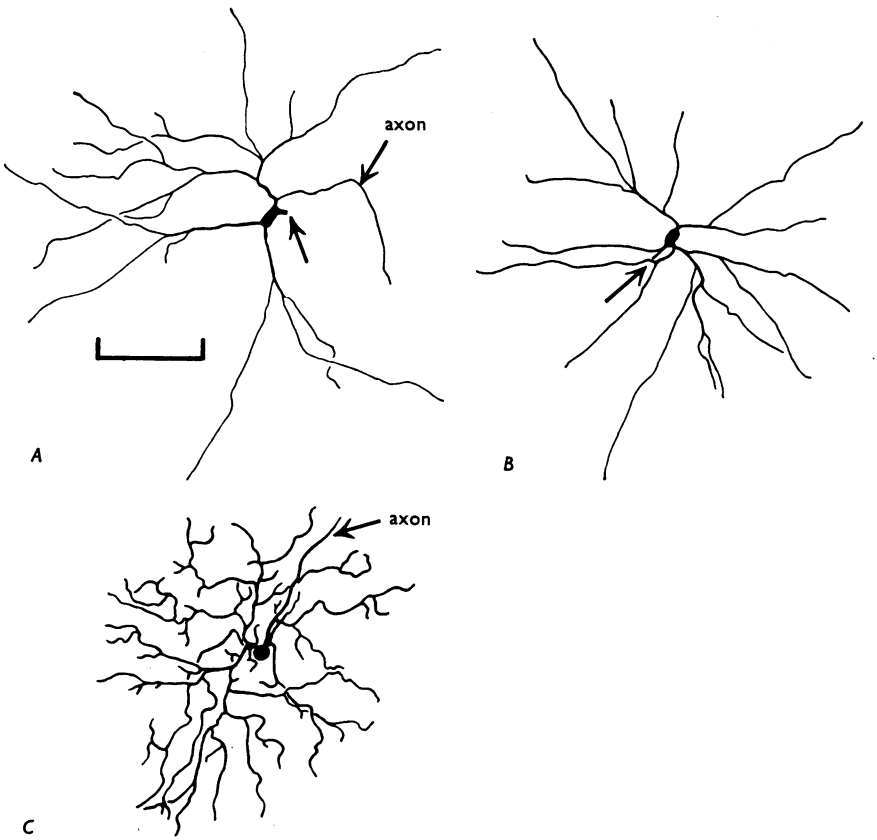
Most of the γ cells are characterized by small, often oval, perikarya. Their primary dendrites rapidly become very thin as soon as they branch. There are usually three or four primary dendrites and generally the dendrites branch much less frequently than the α and β cells as they form the dendritic field. All these cells appear flat because, except in the central area, the primary dendrites come off at the sides of the perikarya and do not seem to run very deeply into the inner plexiform layer. The general morphology of the majority of the γ cells observed is thus very distinctive when compared with that of the α and β cells. This is clearly seen by a comparison of the cells in the illustrations. A few cells were observed, such as those illustrated in Text-fig. 5*C* and Pl. 2, fig.1, which appear somewhat intermediate between the α and γ types. Our interpretation of these cells is discussed in detail on page 412.

Close examination of the dendrites of cells of the type shown in Text-figs. 4*A*, *B* and 5*A*, *B*, may sometimes reveal occasional patches of faintly impregnated small spines in Golgi–Cox prepared material. Such spines were more clearly observed on cells impregnated with Golgi-rapid and Golgi–Colonnier procedures. The spines were less than 0·5 μm in diameter and



Text-fig. 4*A* and *B*. Both these cells are γ-type cells. *A* is at 1·2 mm from the central area and shows a well stained axon. *B* is at 6·3 mm from the central area; an axon was *not* observed on this cell. Although the two cells are at different retinal eccentricities they have very much the same dendritic field diameters and morphologies. In the process of drawing for reproduction, the dendrites on these cells and those of Text-fig. 5 have been somewhat coarsened. A more accurate impression of the observed quality of the dendrites of γ cells is given in Pl. 2, fig. 1 and Pl. 3, fig. 1.

no more than a micrometre in length. Unfortunately they were inadequately impregnated on a sufficiency of cells in any of our Golgi material and therefore their distribution on the dendrites of even one cell, or whether they are present on all the γ cells, is unknown. Text-figs. 13 and 14*B* of Shkolnik-Yarros (1971) give as good a description as we have been able to obtain of the shape and distribution of the dendritic spines on γ cells.



Text-fig. 5*A*, *B* and *C*. *A* and *B* show γ cells at 1·4 and 2·7 mm from the central area. In *A* the axon is coming off a primary dendrite. This cell might be considered understained because of the distinct break in one of the primary dendrites (arrow). Despite the similarity of cell *B* to *A*, including an incompletely stained dendrite (arrow), no axon was observed on it. The cell in *C* is a drawing of the top left cell in Pl. 2, fig. 1, at 2·8 mm from the central area. It shows the contrast of the δ cells with the other γ cells. In respect of the perikaryal size and the thin axon the cell resembles the other γ cells but the branching pattern, although not the thickness of the axons and dendrites, differs subtly from that of the average γ cells.

On all the α and β cells examined, an axon (defined as a process that leaves the perikaryon or a dendrite of a cell, passes out of the plane of the ganglion cell perikaryon layer into the optic nerve fibre layer, and is stained so that it can be seen to go at least some distance in that layer towards the optic disk) has been identified. Only some of the γ-type of cells have shown an axon. Because the general form of these cells resembles that of some of the amacrine cells observed in the cat's retina (B. B. Boycott unpublished), and in other mammalian retinae (Cajal, 1892), it has to be considered that these cells might be displaced amacrine cells. The term displaced amacrine cell is often used (Cajal, 1892) to subsume amacrine cells with perikarya within the inner plexiform layer, as well as amacrine cells with their perikarya in the ganglion cell layer. The former, perhaps best called interstitial amacrine cells (Boycott & Dowling, 1969), are quite common in the retina of the ox (Cajal, 1892, 1911). The latter apparently have not been described in mammals, except for some cells mentioned by Gallego (1971) from the peripheral retina of the cat and the dog. In the cat, what are apparently the same kind of cells as Gallego's were regarded by Leicester & Stone (1967) as having their cell bodies within the inner plexiform layer. Yet displaced amacrines, with their perikarya in the ganglion cell-body layer, are a generally accepted feature of amphibian, reptilian and avian retinae (Cajal, 1911; Polyak, 1957) and may indeed, at least in birds (Binggeli & Paule, 1969), be rather numerous.

In the three Golgi–Cox preparations used, thin axons were found coming from the perikaryon of eighteen γ cells. Another seven γ cells had axons arising from the dendrites as illustrated in Text-fig. 5*A*. Such an origin for an axon from retinal ganglion cells of other mammals has been described by Cajal (1892) and Polyak (1941); and in pl. 43, fig. 86 of Boycott & Dowling (1969). Thus there were axons on twenty-five out of about 150 cells, the morphology of those processes defined them as γ type. In view of the fact that these axons are very thin and likely to be difficult to stain, we have proceeded on the assumption, while wishing to emphasize the reservations given above, that all the cells of the γ-type morphology as defined here possess an axon and are, therefore, ganglion cells.

(*c*) *Ganglion cells in the central area.* Within the central area of the cat's retina Leicester & Stone claimed ganglion cells that had very small dendritic field diameters and perikaryal diameters of between 10 and 15 μm. We have been unable to find ganglion cells in the cat's retina with dendritic field diameters of less than 20–25 μm, which is a good deal larger than Leicester & Stone's statements imply. It is probable that Leicester & Stone were describing understained cells. In addition it must be emphasized that the central area is not exclusively populated by small-field β cells, although these are probably very numerous. Alpha and γ cells with dendritic

fields at least as big as 105–200 μm have been found in the cat's central area (Text-figs. 7 and 8).

(*d*) *Other kinds of ganglion cells.* In general because Shkolnik-Yarros (1971) used only vertical sections, it is difficult to match the data she gives with ours, and with those of Brown & Major (1966) and Leicester & Stone (1967). Also she used both kittens and cats without discriminating between the two in her presentation; perhaps this is why the dimensional data for her types overlap. It is likely that Shkolnik-Yarros's 'miniature', 'small' and some of her 'medium' cells correspond to our β cells and her 'larger' cells to our α cells. Those which certainly match with our γ cells are discussed on page 406.

We have observed one kind of cell in the ganglion cell layer that is clearly different from all the ganglion cell types so far described (Pl. 3, fig. 2). A similar cell was interpreted by Cajal (1891 and 1892, in vertical sections of the dog's retina, Pl. 5, fig. 9*b*) as a ganglion cell; although he did not describe it as with an axon. Such cells were frequently seen partially stained in our Golgi–Cox material, or obscured by precipitation or pieces of Müller's cells. Only three cells were clearly stained, their perikaryal dimensions were 15 μm and the field occupied by the processes was about 300–400 μm. As Pl. 3, fig. 2 shows, the processes of the cells, because they are fine, resemble the dendrites of the γ cells more than those of the α and β cells but their branching pattern resembles none of these three classes. There were no indications of an axon. With so little data a decision could not be made as to whether this type of cell is a ganglion, a glial (it is suggestive of an astrocyte) or even a displaced amacrine cell.

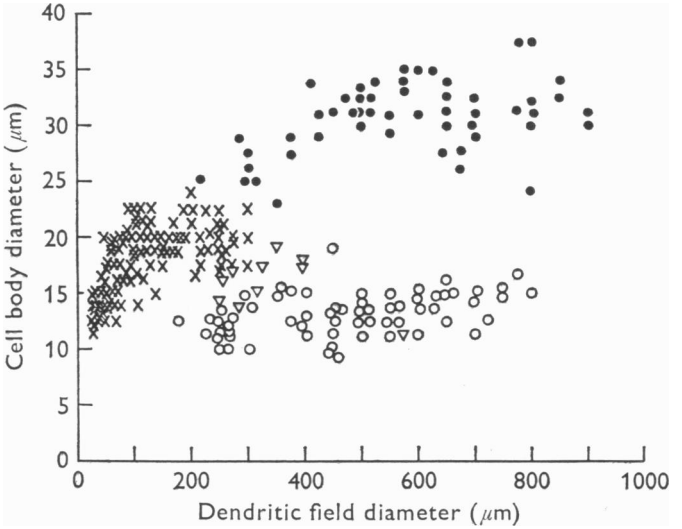
Rushton (1949) and Gallego (1965) described ganglion cells in the cat's retina which stained with reduced silver methods of the Bielschowsky type. The cells revealed with these neurofibrillar stains by Gallego and also Leicester & Stone (1967) and Honrubia & Elliott (1970) have a general morphology that resembles our α-type cell. Within the limits of comparison possible (page 401) the perikaryal dimensions of these cells fall into the α-grouping in our Text-fig. 6. The dimensions for the dendritic fields given in Gallego, and the measurements we made from the sixth figure in Honrubia & Elliott, show that these cells are all distinctly larger than our β-category but at equivalent eccentricities on the retina are smaller than our α cells. Thus there is a possibility that these may be a population of cells, morphologically resembling our α cells, which have not been stained by Golgi procedures. However, because the gross branching pattern is very similar to the α cells, it is only with respect to the dimensions of their dendritic fields that such doubts arise. In order that ganglion cells stained with reduced silver shall be seen through the thickness of whole mounts of the retina and the background darkening that occurs during staining, the pH and toning procedures have to be adjusted very carefully. These adjustments may result in the finer processes of the dendrites being unstained or, especially in whole mounts, unresolvable (B. B. Boycott, unpublished; A. Gallego, personal communication). It is likely, therefore, that the published dimensions of the dendritic field diameters of reduced silver-stained ganglion cells are underestimated and that they belong to our α category.

Neurofibrillar staining methods (Gallego & Cruz, 1965; Honrubia López, 1966) have also revealed, in human and canine retinae, ganglion cells whose morphology resembles those described above but whose axons branch several times, ramify across, and end within the retina. We have not seen such cells in the cat and Gallego (1965 and personal communication) has been unable to find them using his methods. Nor have we seen collaterals, as described by Marenghi (1900), on the axons of any of the ganglion cell types.

All authors are agreed that in the cat's retina there are no bistratified or multistratified ganglion cells as described by Cajal (1892), Polyak (1941, 1957), Boycott & Dowling (1969) and West & Dowling (1972) in other mammalian retinae. No displaced ganglion cells were found. Indeed, except in primates (Boycott & Dowling, 1969; Polyak, 1941), displaced ganglion cells have not been described in the mammals, although they have often been seen in other vertebrate classes (Cajal, 1892, 1911). Shkolnik-Yarros (1971) described ganglion cells whose dendrites were eccentrically placed with respect to the perikaryon. From her descriptions of their branching pattern, cells of this kind are to be found represented in each of our three classes, and we do not regard them as a special morphological type separate from the categories we are defining in this paper.

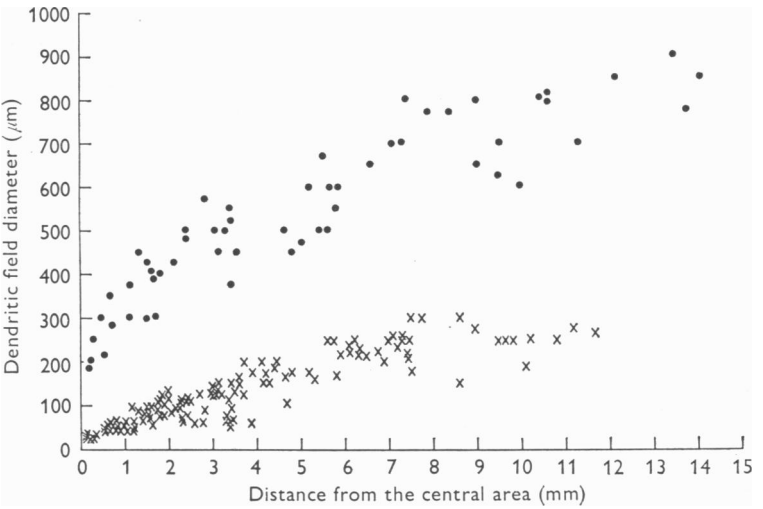
The perikaryal and dendritic field dimensions of alpha, beta and gamma cells and their distribution across the retina

(*a*) *Perikaryal diameter as a function of dendritic field diameter.* Brown & Major (1966) expressed the data for their presumed single type of ganglion cell as a scatter diagram of perikaryal diameter against dendritic field diameter. Within their single type they obtained a distribution showing two populations of dendritic field sizes with ranges which did not overlap. The minimum and maximum dendritic field diameters of their two groups



Text-fig. 6. Perikaryal diameters for cells of the three types of retinal ganglion cell expressed as a function of their dendritic field diameter. The data are from three eyes of two adult cats all stained with Golgi–Cox, and here and in the following graphs ● represents α cells, × the β cells, ○ the γ cells, and ▽ the special category of the γ cells called δ cells. With all these graphs, cells that were widely at variance with their category were re-examined and, if necessary, re-classified. Only 2 % of all the data points could be so treated.

were 70–200 μm and 400–700 μm. These two groups correspond to our β and α types of cells. Text-fig. 6 shows that we found no bimodal distribution within a single cell type (α, β or γ). This figure shows clearly that the three types of cells proposed on qualitative grounds in the preceding section can be justified quantitatively. It is also clear from that figure, when α and γ ganglion cells are compared, that cells with small perikarya (γ cells) can have dendritic fields as large as cells with large perikarya (α cells).

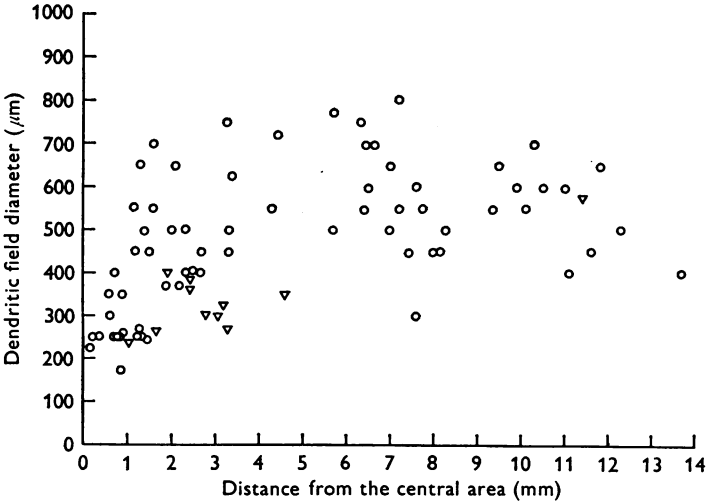


Text-fig. 7. Dendritic field diameters of α and β ganglion cells as a function of distance from the central area. The cells are those used in Text-fig. 6, except for four α cells and nine β cells in the central area that were measured from Golgi-rapid stained material. In this and the succeeding graphs distance is taken in millimetres on the slide; to get degrees of visual angle accurately it would be necessary to account for the distortions due to the staining procedures and flattening of the retina when mounted on the slide. For conventions see Text-fig. 6.

(*b*) *Dendritic field dimensions.* Text-fig. 7 shows for α and β cells that there is a regular progression of increasing dendritic field diameter across the retina. The range of the dendritic field diameters of the α cells in that figure is from 180 μm in the central area, to about 900 μm at 14 mm distance from the central area. However, at approximately 15 mm from the central area, in material other than that used for the graphs, one or two α cells of 1·0 mm diameter have been found. The graph includes no β cells beyond 12 mm from the central area. But because the ganglion cell densities are low in the periphery of the cat's retina (Stone, 1965) and because of the uncontrollabilities of the Golgi-staining, it cannot be concluded

from this evidence that there are no β cells peripheral to 12 mm. We saw a few β cells beyond 12 mm from the central area in material not included in Text-fig. 7; they never had a dendritic field diameter much larger than 300 μm.

Text-fig. 8 shows the dendritic field dimensions of the γ cells as a function of distance from the central area. Point for point across the retina this dimension of the cells is always greater than it is for the β cells (Text-fig. 7). The β cells are thus the class of cells which, at all retinal eccentricities, have the smallest dendritic fields. In terms of dendritic field sizes the γ



Text-fig. 8. Distribution of the dendritic field sizes of γ cells across the retina as a function of distance from the central area. The cells are from the two retinae of the same cat. Conventions the same as Text-fig. 6.

cells scatter around the dendritic field dimensions of the α cells. Although the γ cells have on average the smallest perikaryal dimensions of the three groups of ganglion cells (Text-figs. 6 and 9), at least some of the members of the group, at most retinal eccentricities up to about 8·0 mm from the central area, have dendritic field diameters larger than those of even the α-type cells. As Text-fig. 8 shows, even in the central area the dendritic field sizes of the γ cells, like the α cells, are large. Gamma ganglion cells with fields as large as 200–400 μm can be found within 1·0 mm of the central area. It is clear that there are α and γ cells with dendritic field diameters as large as 150–200 μm within that area.

The γ cells contrast with α and β types in that the variance of the dendritic field diameters of the γ-type grouping is greater at most points across the retina. From the data available (Text-fig. 8) only within and near the

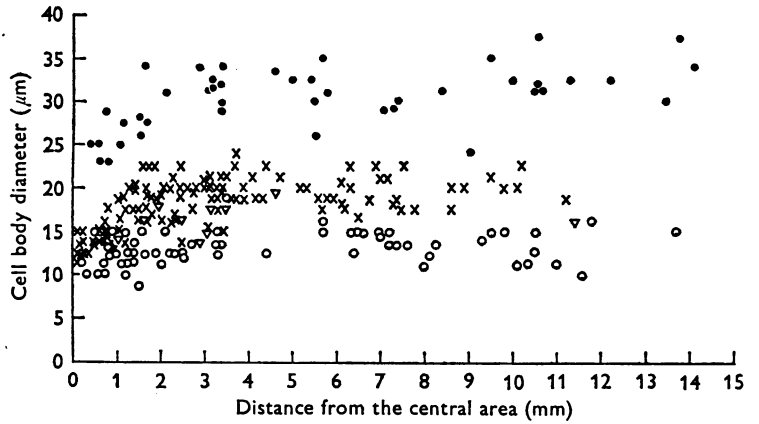
central area is there any indication of increasing diameter with distance towards the periphery. It therefore seems that representative γ cells from the spectrum of small to large dendritic field diameters are to be found at all retinal locations. It must, however, be remembered that the γ cells may represent a morphologically heterogeneous population whose different characteristics are not yet recognized (see below). It is clear from the data in Text-figs. 6 and 9 that they do not represent merely erratically stained α cells.

Although all the perikarya of the γ cells measured were fully stained, we know, as discussed on page 406, that the spines on the dendrites were not regularly impregnated and that the fine axon was only sometimes stained. These three observations did not provide criteria for judgement of the degree to which the dendritic fields were stained. The dendrites of the γ cells are characteristically small in diameter; for most of their length they are as little as 1·0 μm in diameter or less. It is to be expected that they may not always stain to their full extent. In quite a number of cells (included in the data) we felt that we might not have been seeing their full extent (see for example Text-fig. 5*A* and *B*). Our reasons were simply that the pattern of some of the branches of a particular cell seemed to stop short, giving an irregular appearance to the outline of the dendrites. Yet we did not feel that we could justify exclusion of cells of this kind from the data solely because of intuitive reasoning from an ideal of the shape or size of a cell. In any case with many of the γ cells with smaller fields more often than not there was no such reason to justify their exclusion. These uncertainties also mean that γ cells with dendritic field dimensions larger than the maximum of 800 μm so far found could be present in the retina.

A few cells were found within the γ-cell grouping which did seem to have differences in their general morphology and size that suggested a distinctive morphological type. These are the cells marked by the triangular symbols in Text-figs. 6, 8 and 9. Only eleven cells of this kind were found, of which five had axons stained (see page 407). For convenience they may be referred to as the δ cells. On average their perikaryal diameters were larger than those of other cells of the γ class (16 μm as compared to 13 μm, Text-fig. 8). All of them had the general form of those illustrated in Text-fig. 5*C* and Pl. 2, fig. 1. From these figures it can be seen that their dendritic morphology resembles that of the smaller α cells (Text-fig. 2*A*). Indeed the δ cells are easily confused with the α cells until they are compared with that type at specified retinal locations, when they are found to have consistently smaller dendritic field diameters, smaller perikarya, thinner dendrites and thinner axons than the α cells. Although the perikaryal diameters of the δ cells fall within the range of the β cells, their dendritic field diameters are always larger than those of the β cells. We do not feel

that we have seen a sufficient number of the δ cells, or discriminated the possible special features of their morphology clearly enough, to be able confidently to define them as a separate morphological type of ganglion cell.

(*c*) *Perikaryal dimensions.* All the previous data are based on the qualitative recognition of differences in the branching pattern of the dendrites of the three classes of ganglion cells. Text-fig. 9 shows that the perikaryal diameters of α, β and γ cells hardly overlap. Over most of the retina there is no significant increase in perikaryal diameter with distance from the central area for any of the three groups of cells. The means of the perikaryal dimensions of the three classes of ganglion cells beyond 2·0 mm from the



Text-fig. 9. Perikaryal diameter of all classes of ganglion cell as a function of distance from the central area. The nine β cells represented here as within the central area (but not included in Text-fig. 6) are taken from Golgi-rapid material. Because of the effect described in Text-fig. 1 they probably appear too large with respect to all the other points, which are from the same Golgi–Cox stained material as Text-fig. 6.

central area are given in Table 1. Nearer than 2·0 mm from, and within the central area, the α cell perikarya are so large that they remain clearly distinct. The β and γ cell perikarya are dimensionally less distinctive. It is within this region that Stone (1965) has shown a very sharp increase in the density of ganglion cell perikarya per unit area. The packing of a high cell density into this region may be thought to contribute to the blurring of the dimensional distinction between the perikarya of the two populations. There is, however, a further factor hidden by our method of measurement (page 399). Some of the γ cells have oval perikarya with the long axis of their perikarya parallel to the plane of the retinal surface. For such cells the ratio of the axes was about 3:1. Because the dendrites came off the side of the perikaryon (Text-fig. 5) exact judgement of the extent of the long axis was difficult. Thus the larger dimensions of some of the γ cell's

perikarya may have been an overestimation. Among the γ-cell grouping this comment does not apply to the δ cells, because their perikarya are usually spherical. As with their other characteristics the data are too few for them definitely to be excluded from the γ-cell grouping.

DISCUSSION

The data presented here are the first to show anatomically for any vertebrate the relationship of some of the dimensions of different morphological types of ganglion cell with retinal eccentricity. As the graphs show, each morphological type is represented at all locations of the retina, certainly up to 12 mm from the central area. There appears to be no significant retinal region where one morphological type of ganglion cell is represented to the exclusion of another; even within the central area all three morphological types of cell have been found. This is not to say that the proportions of the different types of cells do not differ at different retinal eccentricities. Stone (1965), observing the perikaryal dimensions of the cat's retinal ganglion cells, showed that the number per unit area of all classes of ganglion cell perikarya was much greater in the central area than in the periphery. And, particularly in the central area, the number of the smaller ganglion cell perikarya was relatively greater than the number of larger ganglion cell perikarya. The perikaryal dimensions of the α cells in our Golgi–Cox material are between 23 and 35 μm. Assuming that these correspond to the large perikarya (21 μm and above) in Stone's data, then there are more β and γ cells relative to α cells in the central area. Unfortunately the material is not yet available for it to be possible to give more precise anatomical estimates of the proportions, or changes in the proportions across the retina, of the different types of ganglion cells.

In the presentation of the results no descriptions of the axons and their diameter were given. Approximately speaking it appeared that the larger the perikaryon the larger was the axonal diameter. Detailed measurements of axonal diameters on the kind of material used would have been insufficiently precise due to irregular distortions of the axons along their stained length and the different responses of the cells to Golgi-rapid and Golgi–Cox staining (page 400). Some of the axonal swellings are possibly a feature of the axonal structure since they have been observed by electron microscopy (Stone & Holländer, 1971). Despite the lack of quantitative information it must be emphasized that subjectively the diameters of the axons of α cells were, at equivalent retinal points, larger than the axons of the β and γ cells. Similarly the β cells had larger axonal diameters than those γ cells on which an axon could be observed. There was also an impression that for the α and β cells the diameters of the axons of cells situated in the central

area were smaller than for those a millimetre or two outside the central area.

There have been previous attempts in mammals to correlate physiological and morphological types of ganglion cells. West & Dowling (1972) measured the ratios of amacrine and bipolar cell synapses on to different morphological types of ganglion cells in the ground squirrel's (*Citellus*) retina. They correlated a high bipolar cell input with those ganglion cells which show 'tonic' behaviour and a high amacrine input with 'phasic' behaviour. They suggested that the large number of morphological types of ganglion cells in the ground squirrel's retina may not be reflected in an equivalent variety of physiological types. Brown (1965) suggested a correlation between the two anatomical types of ganglion cells he described in the retina of the white rat and the observation that in this retina there are on- and off-centre cells with opponent surrounds, and a second group with on- or off-centres but no opponent surrounds (Brown & Rojas, 1965).

Observations that simplify the attempt to suggest morphological and physiological correlations in the cat are provided by Cleland *et al.* (1971, 1973). They have shown that on-centre and off-centre cells divide into X and Y classes in about equal numbers. They have also shown that in the X class, receptive field centre sizes are equally distributed between the on and off-centre cells. For the Y class, however, they found that the off-centre cells on the average have larger receptive field centres than the on-centre cells. These were not, however, two clearly separated groupings, since the dimensions overlapped. Unfortunately, their sample was small and lacked information as to the retinal eccentricity of the cells. However, off-centre and on-centre ganglion cells did not fall into two separate classes according to conduction velocity, although the X and the Y classes did so differ. From these data it follows that an initial attempt to correlate ganglion cell types and physiological classes should be with X and Y cells, not with on-centre and off-centre cells.

Table 1 summarizes our anatomical information and the information of those authors who make the dichotomy into X and Y cells. All authors are agreed that the receptive field centres of the Y cells are larger than those of the X cells (Enroth-Cugell & Robson, 1966; Fukada, 1971; Cleland *et al.* 1971, 1973; Ikeda & Wright, 1972*b*), and the Y cells' axons have the fastest conduction velocities to the dorsal lateral geniculate body and the superior colliculus (Cleland *et al.* 1971, 1973; Hoffmann *et al.* 1972; Stone & Hoffmann, 1972 and Hoffmann, 1973). Even considering their total receptive field sizes the X cells have smaller fields than the Y cells (Cleland *et al.* 1973). Our paper shows that the α cells have larger dendritic fields and larger axons than the β cells at equivalent retinal loci. It is therefore clear that the most likely anatomical basis for the X cells is within the β

ganglion cell category. The discovery that the γ-type ganglion cells may have dendritic field sizes as large as the α-type ganglion cells at first sight confuses a correlation of the α-type cells with the Y cells. But the γ cells have the smallest axons and therefore, presumably, the slowest conduction

TABLE 1. Anatomical and physiological dimensions of cat retinal ganglion cells

Anatomical dimensions			
Cell type ...	α	β	γ
Dendritic field size (μm)	180–1000	20–300	180–800
Dendritic field size (deg)	0·8–4·5	0·1–1·4	0·8–3·6
Perikaryal diam. (μm)	23–38	11–24	8–18
*Mean perikaryal diam. μm	32·5	20	13·5
Axon diameter	Thick	Medium	Thin

Physiological dimensions of receptive field centres			
Cell type ...	Y	X	W
Authority			
Enroth-Cugell & Robson (1966)	1–7°	0·55–2·9°	—
Fukada (1971)	1·5–6·5°	0·5–4·5°	—
Ikeda & Wright (1972*b*)	1·0–3·0°	0·4–0·8°	—
†Cleland, Levick & Sanderson (1973)	0·5–1·9°	0·22–0·81°	—
Conduction velocity (m/sec) in the optic nerve			
Fukada (1971)	20–60	10–40	—
Hoffmann, Stone & Sherman (1972)	36–44	19–24	—
Stone & Hoffmann (1972)	—	—	5·5–14
Hoffmann (1973)	35–45	—	< 15
Conduction time (msec)			
Cleland, Dubin & Levick (1971)			
Retinal ganglion cell–dorsal lateral geniculate cell	1·8–3·0	3·4–8·8	—
Hoffmann, Stone & Sherman (1972)			
Optic chiasm-dorsal lateral geniculate cell	0·9–1·7	1·5–3·1	—

* Excluding a circle of 2 mm radius from the central area (see page 413).
† These data extend only to 30° from the central area.

velocities; which is what the W cells have. The γ cells all have small perikarya and so they are the cells least likely to have been recorded from until recently (Wiesel, 1960). Thus the most likely correlation is between the α and Y cells and the γ and W cells.

From the Table it can also be seen that the dimensions of the receptive field centres of the X and Y cells are similar to the dendritic field diameters of the β and α cells. The exact relationship between the two still remains unclear.

During the past year our results have been available to B. G. Cleland and W. R. Levick whose two papers are on pages 421 and 457 of this Journal, and to Y. Fukuda and J. Stone whose two papers are in the *Journal of Neurophysiology*, July 1974. Based on their new physiological data these authors provide fuller discussions of possible anatomico-physiological correlations.

Many friends and colleagues in Cambridge, Canberra, Boston, London and München have commented on this paper while it was in preparation. We thank them all and accept blame for its remaining sins of omission and commission. H. Wässle was supported by a training grant of the European Brain and Behaviour Society, and by the Deutsche Forschungsgemeinschaft, SFB, 50. We thank Mr Z. Gabor for the photomicrography and Mr J. M. Hopkins for assistance in many capacities.

REFERENCES

BINGGELI, R. L. & PAULE, W. J. (1969). The pigeon retina: quantitative aspects of the optic nerve and ganglion cell layer. *J. comp. Neurol.* **137**, 1–18.

BISHOP, P. O., KOZAK, W. & VAKKUR, G. F. (1962). Some quantitative aspects of the cat's eye: axis and plane of reference, visual field co-ordinates and optics. *J. Physiol.* **163**, 466–502.

BOYCOTT, B. B. & DOWLING, J. E. (1969). Organization of the primate retina: light microscopy. *Phil. Trans. R. Soc.* B **255**, 109–184.

BOYCOTT, B. B. & KOLB, H. (1973*a*). The connexions between bipolar cells and photoreceptors in the retina of the domestic cat. *J. comp. Neurol.* **148**, 91–114.

BOYCOTT, B. B. & KOLB, H. (1973*b*). The horizontal cells of the rhesus monkey retina. *J. comp. Neurol.* **148**, 115–139.

BROWN, J. E. (1965). Dendritic fields of retinal ganglion cells of the rat. *J. Neurophysiol.* **28**, 1091–1100.

BROWN, J. E. & MAJOR, D. (1966). Cat retinal ganglion cell dendritic fields. *Expl Neurol.* **15**, 70–78.

BROWN, J. E. & ROJAS, J. A. (1965). Rat retinal ganglion cells: receptive field organization and maintained activity. *J. Neurophysiol.* **28**, 1073–1090.

CAJAL, S. R. (1891). Sobre la retina y gran simpatico de los mamíferos. *Notas Preventivas*. Barcelona: Gaceta Sanitaria.

CAJAL, S. R. (1892). *Die Retina der Wirbelthiere*. Wiesbaden: Bergman; trans. THORPE, S. A. & GLICKSTEIN, M. (1972). *The Structure of the Retina*. Springfield, Illinois: C. C. Thomas.

CAJAL, S. R. (1911). *Histologie du Système Nerveux de l'Homme et des Vertébrés*, vol. **2**. Paris, A. Maloine. Reprinted 1952. Madrid: Consejo superior de investigaciones cientificas, Instituto Ramõn y Cajal.

CLELAND, B. G., DUBIN, M. W. & LEVICK, W. R. (1971). Sustained and transient neurones in the cat's retina and lateral geniculate nucleus. *J. Physiol.* **217**, 473–496.

CLELAND, B. G., LEVICK, W. R. & SANDERSON, K. J. (1973). Properties of sustained and transient ganglion cells in the cat retina. *J. Physiol.* **228**, 649–680.

CREUTZFELDT, O. D., SAKMANN, B., SCHEICH, H. & KORN, A. (1970). Sensitivity distribution and spatial summation within receptive-field center of retinal on-center ganglion cells and transfer function of the retina. *J. Neurophysiol.* **33**, 654–671.

DOWLING, J. E. & BOYCOTT, B. B. (1966). Organization of the primate retina: electron microscopy. *Proc. R. Soc.* B **166**, 80–111.

ENROTH-CUGELL, C. & ROBSON, J. C. (1966). The contrast sensitivity of retinal ganglion cells of the cat. *J. Physiol.* **187**, 517–552.

FUKADA, Y. (1971). Receptive field organization of cat optic nerve fibers with special reference to conduction velocity. *Vision Res.* **11**, 209–226.

FUKADA, Y. & SAITO, H. A. (1971). The relationship between response characteristics to flicker stimulation and receptive field organization in the cat's optic nerve fibers. *Vision Res.* **11**, 227–240.

GALLEGO, A. (1965). Connexions transversales au niveau des couches plexiformes de la rétine. *Actualités Neurophysiologiques*, 6e sér., pp. 5–27. Paris: Masson et Cie.

GALLEGO, A. (1971). Horizontal and amacrine cells in the mammal's retina. *Vision Res.* suppl. **3**, 33–50.

GALLEGO, A. & CRUZ, J. (1965). Mammalian retina: associational nerve cells in ganglion cell layer. *Science, N.Y.* **150**, 1313–1314.

HOFFMANN, K. P. (1973). Conduction velocity in pathways from retina to superior colliculus in the cat: a correlation with receptive-field properties. *J. Neurophysiol.* **36**, 409–424.

HOFFMANN, K. P., STONE, J. & SHERMAN, S. M. (1972). Relay of receptive-field properties in dorsal lateral geniculate nucleus of the cat. *J. Neurophysiol.* **35**, 518–531.

HONRUBIA LÓPEZ, F. M. (1966). Estudio de los campos anatómicos de las células ganglionares de la retina. *Arch. Soc. oftal. hisp.-amer.* **26**, 693–720.

HONRUBIA, F. M. & ELLIOTT, J. H. (1970). Dendritic fields of retinal ganglion cells in the cat. *Archs Ophthal., N.Y.* **84**, 221–226.

IKEDA, H. & WRIGHT, M. J. (1972*a*). Functional organization of the periphery effect in retinal ganglion cells. *Vision Res.* **12**, 1857–1879.

IKEDA, H. & WRIGHT, M. J. (1972*b*). Receptive field organization of 'sustained' and 'transient' retinal ganglion cells which subserve different functional roles. *J. Physiol.* **227**, 769–800.

KUFFLER, S. W. (1952). Neurons in the retina: organization, inhibition and excitation problems. *Cold Spring Harb. Symp. quant. Biol.* **17**, 281–292.

KUFFLER, S. W. (1953). Discharge patterns and functional organization of mammalian retina. *J. Neurophysiol.* **16**, 37–68.

LEICESTER, J. & STONE, J. (1967). Ganglion, amacrine and horizontal cells of the cat's retina. *Vision Res.* **7**, 695–705.

MARENGHI, G. (1900). Contributo alla fina organizzazione della retina. *Anat. Anz.* **18**, 12–16.

POLYAK, S. L. (1941). *The Retina.* Chicago: University of Chicago Press.

POLYAK, S. L. (1957). *The Vertebrate Visual System.* Chicago: University of Chicago Press.

RODIECK, R. W. (1967). Maintained activity of cat retinal ganglion cells. *J. Neurophysiol.* **30**, 1043–1071.

RUSHTON, W. A. H. (1949). The structure responsible for action potential spikes in the cat's retina. *Nature, Lond.* **164**, 743–744.

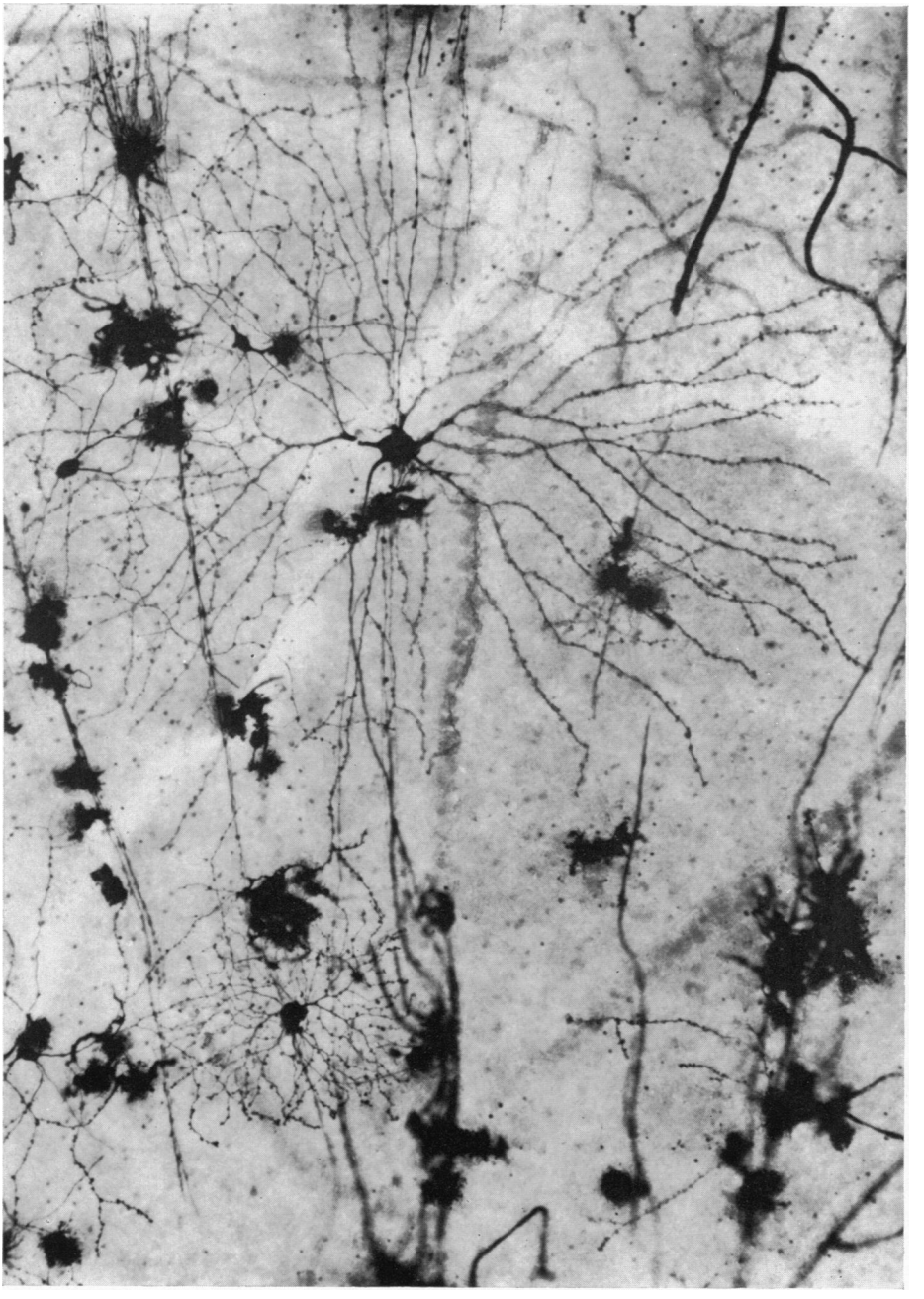
SHKOLNIK-YARROS, E. G. (1971). Neurons of the cat's retina. *Vision Res.* **11**, 7–26.

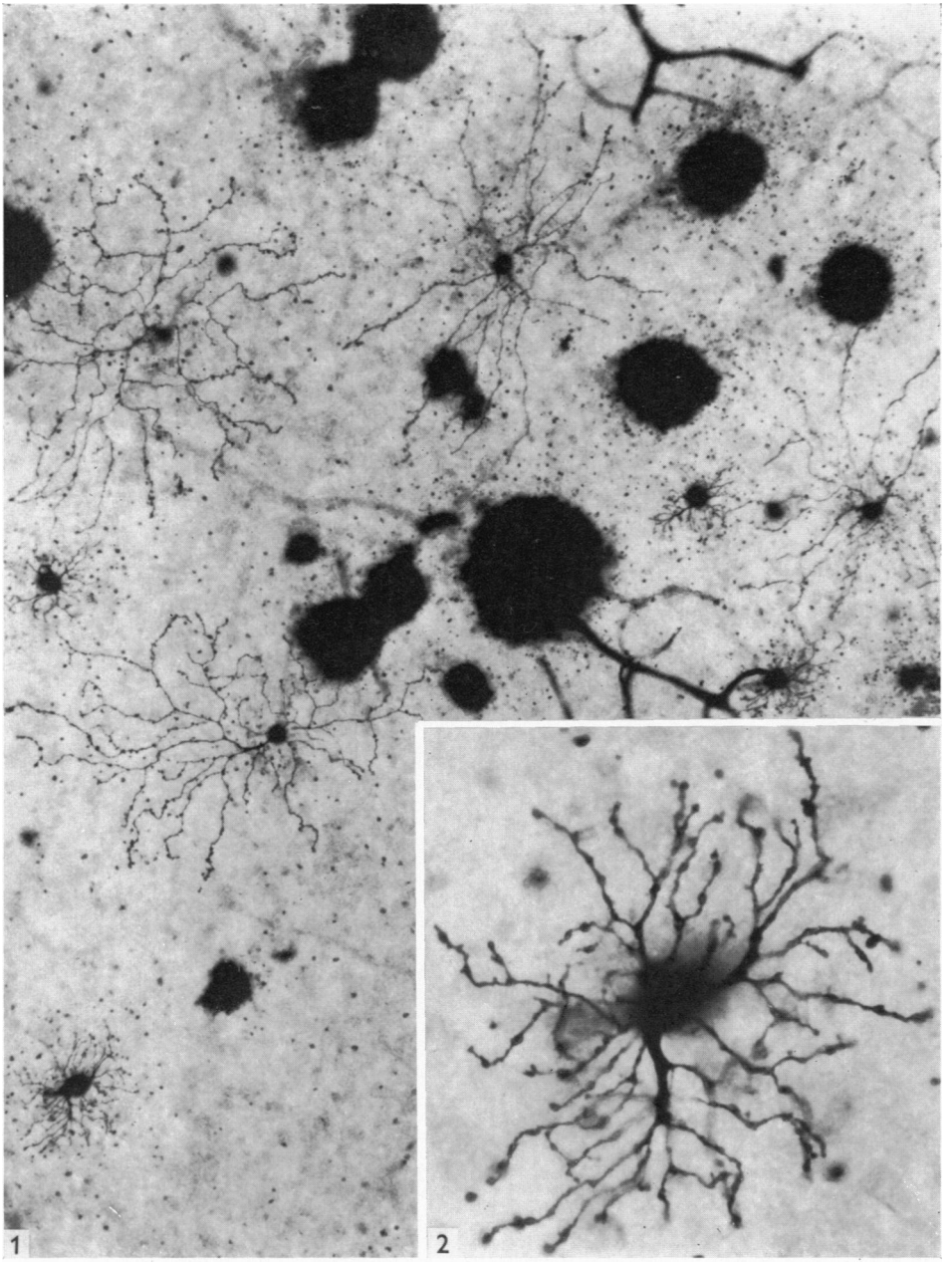
STONE, J. (1965). A quantitative analysis of the distribution of ganglion cells in the cat's retina. *J. comp. Neurol.* **124**, 337–352.

STONE, J. & FABIAN, M. (1966). Specialized receptive fields of the cat's retina. *Science, N.Y.* **152**, 1277–1279.

STONE, J. & HOFFMANN, K. P. (1972). Very slow-conducting ganglion cells in the cat's retina: a major new functional type. *Brain Res.* **43**, 610–616.

STONE, J. & HOLLÄNDER, H. (1971). Optic nerve axon diameters measured in the cat retina: some functional considerations. *Expl Brain Res.* **13**, 498–503.

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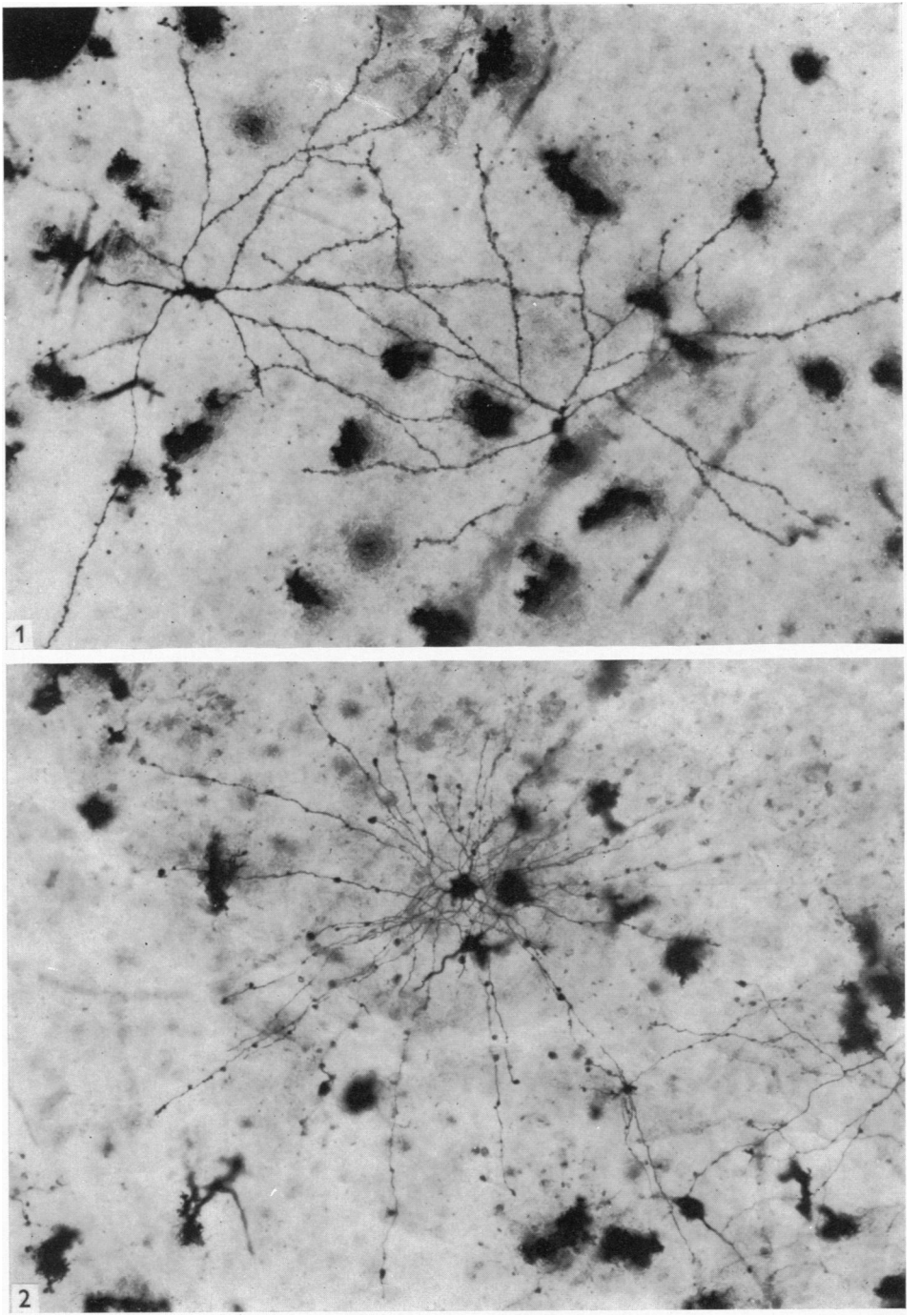
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WEST, R. W. & DOWLING, J. E. (1972). Synapses onto different morphological types of ganglion cell. *Science, N.Y.* **178**, 510–512.

WIESEL, T. N. (1960). Receptive fields of ganglion cells in the cat's retina. *J. Physiol.* **153**, 583–594.

EXPLANATION OF PLATES

PLATE 1

Alpha (top) and β (lower) ganglion cells 7·4 mm from the central area to show the contrast between the morphologies of the dendrites of the two types of cell. Other cells with the smallest perikarya are γ ganglion cells. The bundles of fibres are optic nerve fibres. The large black clots are pieces of stained Müller's cells. The black dots on the unstained tissue and along the nerve cell processes are a precipitate that seems to be a concomitant of the reaction of the retina to Golgi–Cox. Magnification × 160.

PLATE 2

Fig. 1. Piece of retina at the same magnification as Pl. 1. The cells with the smallest dendritic fields are β cells, that on the bottom left is enlarged to × 600 in fig. 2. The two cells, slightly out of focus, top centre and right are γ ganglion cells. The two γ cells in sharp focus on the left are the δ cell variety of the γ cells. The large black blobs are precipitate in traces of vitreous humour. Other comments as for Pl. 1 and distance from the central area 2·8 mm.

PLATE 3

Fig. 1. Two γ ganglion cells at 1·5 mm from the central area. That on the left had an axon, which here is out of focus, coming from the perikaryon. That on the right had no visible axon stained. Other comments and the magnification as in Pl. 1. Fig. 2, a cell of undetermined nature at an unknown distance from the central area (for details see page 408). Magnification × 320. Dendrites at the bottom right are from several γ cells.